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Evolution of microbiological and chemical parameters during red wine making with extended post-fermentation maceration

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ABSTRACT

The aim of the present work was to investigate the microbiological, chemical, and sensory characteristics of red wine subjected to post-fermentation maceration that was extended to 90 days. For this purpose, the 'Aglianico di Taurasi' grape was used as a case study. The total yeast concentration increased until day 40 of maceration and decreased thereafter, whereas the concentration of lactic acid bacteria slightly increased. *Dekkera/Brettanomyces* spp. and acetic acid bacteria were not detected. The yeast community was composed of *Saccharomyces cerevisiae*, *Zygosaccharomyces bisporus*, *Metschnikowia pulcherrima*, *Hanseniaspora guilliermondii*, *Hanseniaspora uvarum*, *Pichia guilliermondii*, *Aureobasidium pullulans* and *Debaryomyces carsonii*. Nine *S. cerevisiae* strains were detected at high levels at different times of maceration.

The results of all the conventional chemical analyses of the wines were in agreement with the regulations of commercial production and, interestingly, the changes in terms of concentration demonstrated the presence of yeast and LAB populations that were not only alive but also in a metabolically active state until day 90 of maceration. The alcohol and glycerol contents slightly increased until day 90. The concentrations of malic acid decreased, whereas those of lactic acid slightly increased throughout the maceration process.

Furthermore, different durations of maceration resulted in significant differences in the total polyphenol content, which was higher at 40–50 days. The main phenolic compounds were benzoic and cinnamic acids and catechins. Interestingly, the highest ratio between (+)-catechin and (−)-epicatechin was found on day 40. In addition, the highest antioxidant activity was observed between days 40 and 50. The concentration of volatile organic compounds, which were mainly represented by alcohols, increased until the end of the maceration process. Sensory analysis revealed that samples that were subjected to maceration for a long period of time showed the highest odour and taste complexity and no off-odours and/or off-flavours were detected. These data confirmed that extending post-fermentation maceration to 90 days has no negative impact on the microbiological, chemical and sensory composition of wines, but affects the polyphenol content and potential health benefits of the resulting wine.

1. Introduction

The 'Aglianico di Taurasi' grapevine is one of the economically most important cultivars of the Campania region (Pomarici et al., 2004). The quality of the resulting wine—the highest category is 'Taurasi D.O.C.G.'—is well recognised (Piombino et al., 2004) and its composition varies significantly depending on several factors, from agronomic practices to the technology of vinification (Mazzei et al., 2010).

Rate, kinetics, and duration of fermentation strictly depend on the yeast population present in the must (Zambonelli, 1998). The metabolic activities of yeast on must components determine the production of several compounds that significantly contribute to the aroma of wines (Pretorius, 2000). An important step in the production of red wine is represented by the maceration process, whose effects influence the quality of the wine (Bautista-Ortín et al., 2005). The main purpose of maceration is the extraction of colour compounds (anthocyanins and phenolic substances) from the solid components of the grape. However, this process also affects the sensory profile of the resulting wines because other compounds such as aromatic substances and precursors, nitrogen compounds, polysaccharides, and minerals are released during the maceration process (De Beer et al., 2006). The transfer of these compounds from grape skins and seeds to the must is influenced by several factors, including temperature, contact duration, alcohol content, SO_2 concentration, grape variety, maturation degree, and microbial populations (Romero-Cascales et al., 2005).

Four maceration techniques may be applied during wine production: conventional maceration, consisting of moving the must from the bottom of the vat to the top or immersing the floating layer of skins in the

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fermenting bulk or by transferring the entire liquid phase of the must from one vat to another one (délestage); carbonic maceration, which is carried out with whole grapes fermented in a CO_2-rich environment; pre-fermentation maceration or "cold soaking", in which grape skins and seeds are put in contact with the liquid must at low temperatures one or two days before fermentation begins and the must is moved a few times per day; and post-fermentation maceration of variable duration, i.e. a few days to 21 days (Ivanova et al., 2011; Gambuti et al., 2004).

The prolonged contact between grape skins and seeds with the must allows a higher extraction of polyphenolic compounds, especially catechins and proanthocyanidins (or condensed tannins) that are more concentrated in the pulp of grape berries (Ivanova et al., 2011). Longer macerations also provide stability of the colour compounds (Gómez-Plaza et al., 2002). Among these, polyphenols are gaining interest due to their positive effects (antioxidant, anticancer, cardioprotective, antimicrobial, antiviral, and neuroprotective) on the consumers' health (En-Qin et al., 2010).

The main objective of the present work was the evaluation of the influence of long post-fermentation maceration on the evolution of yeast and lactic acid bacteria (LAB) populations, release of polyphenol compounds from skins and seeds, volatile organic compounds (VOCs), and antioxidant activity of red wine. The 'Aglianico di Taurasi' cultivar was used as model system.

2. Materials and methods

2.1. Experimental winemaking and sample collection

The experimental winemaking was carried out by prolonging the contact between solid (grape skins) and liquid (wine) phases of grape must to 90 days after the tumultuous phase of alcoholic fermentation. This was considered an extension of the post-fermentation maceration period.

The grapes of the 'Aglianico di Taurasi' grapevine were subjected to the experimental vinification process that took place at the winery 'Azienda Agricola Contrade di Taurasi' located in Taurasi (Avellino, Campania, Italy) (41°00′11.94″N; 14°58′25.82″E).

Soon after harvest, the grapes were subjected to stemmer-crushing. After placing the must into steel vats and adding potassium metabisulphite (6 g/hL), it was inoculated with the autochthonous starter strain *Saccharomyces cerevisiae* NF66 (culture collection of the Department of Agricultural and Forest Science — University of Palermo, Italy) (15 g/hl). The starter (used as paste) was characterised by a viable cell concentration of 7.6×10^{12}-colony forming units (CFU)/g. Diammonium phosphate and diammonium sulphate salts (1:1) (15 g/hL) were also added as activators of the fermentation process. Eighty hectolitres of must were transferred into two stainless steel vats (40 hL each) where the fermentation (8 days at 26 °C) took place. During the tumultuous phase of alcoholic fermentation but only after raising the cap, the content of each vat was mixed in order to facilitate the contact between the solid and liquid phases of the must. In particular, this was done three times per day in order to remove the liquid phase from the bottom of a single vat to the top of the same vat. Furthermore, from day 4 until day 8 of alcoholic fermentation, each vat was subjected to one délestage per day. This was done by transferring the entire liquid phase of the must of each vat into empty stainless steel vats, letting them stand for 4 h, and transferring the liquid phase back into the original vat. The scope of this action was to facilitate the contact between the liquid phase of the must with oxygen.

At the end of the tumultuous phase of alcoholic fermentation (day 8), the bulk content (both liquid and solid phases) of each vat was transferred into steel vats with a capacity of 5 hL. All vats were filled until a final solid-to-liquid ratio of 1:3 was reached and closed to avoid contact with oxygen. They were cooled at 16 ± 1 °C and subjected to different durations of post-fermentation maceration: 13 d, which represented the control of post-fermentation maceration, 20, 40, 50, 60, 70 and 90 d. The production of commercial 'Aglianico di Taurasi' wine after the tumultuous phase of alcoholic fermentation is, in general, based on a maceration period of 13 d, which is considered to be the minimal duration of maceration for this wine. Thus, in this study, post-fermentation maceration of 13 d was used as control trial. The vinification process, i.e., from grape must until day 90 of post-fermentation maceration, was performed in duplicate.

2.2. Microbiological analysis

Must samples collected from grape must until the end of maceration were serially diluted in Ringer's solution (Sigma-Aldrich, Milan, Italy). Decimal dilutions were spread-plated (0.1 mL) onto Wallerstein Laboratory (WL) nutrient agar (Oxoid, Basingstoke, UK) and incubated at 28 °C for 48–72 h to determine total yeast (TY) counts. The sample dilutions were also spread-plated onto *Dekkera*/*Brettanomyces* differential medium (Rodrigues et al., 2001) and incubated at 25 °C for 14 d to detect presumptive *Dekkera*/*Brettanomyces* spp.

The *Dekkera*/*Brettanomyces* population was also counted by filtering (0.45-μm pore size filter, Sartorius, AubagneCedex, France) the samples using the same media and incubation conditions reported above. To count the lactic acid bacteria (LAB), the sample dilutions were pour-plated onto Man, Rogosa, and Sharpe (MRS) agar (Oxoid) and incubated at 28 °C for 48–72 h, and onto medium for *Leuconostoc oenos* (MLO) agar (Caspritz and Radler, 1983) and incubated at 28 °C for 5 d. The latter medium was used for the enumeration of acidophilic LAB. The acetic acid bacteria (AAB) population was enumerated onto Kneifel agar medium (OIV, 2010) and incubated at 25 °C for 10 d. All analyses were carried out in duplicate.

2.3. Yeast isolation and identification

Yeasts were isolated only from WL differential medium. At least five colonies per morphology were randomly collected from the agar plates, purified to homogeneity after several sub-culturing steps onto WL, and at least three isolates (from each sample) sharing the same morphology were subjected to genetic characterisation.

DNA extraction was performed using the InstaGene Matrix kit (Bio-Rad Laboratories, Hercules, CA) according to the manufacturer's instructions. In order to perform a first differentiation of yeasts, all selected isolates were subjected to restriction fragment length

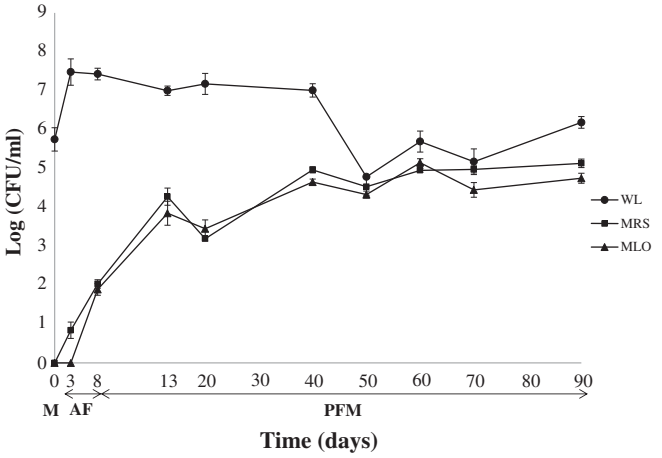

Fig. 1. Microbial loads of wine samples collected during experimental vinification of "Aglianico di Taurasi" wine. Symbols: ●, TY on WL; ■, LAB on MRS; ▲, LAB on MLO. Data represent the mean of four replicates of two independent experiments. Bars represent standard deviation of the mean. Vertical bars not visible are smaller than symbol size. Abbreviations: AF, alcoholic fermentation; PFM, post-fermentation maceration.

Table 1
Molecular identification of yeasts.

R.P.	Isolate code	5.8S	Size of restriction fragments			26S
		ITS	*Cfo*I	*Hae*III	*Hinf*I	PCR
		PCR				
I	TLMBRL119A	620	180 + 110	480 + 160	280 + 160 + 130	1100
II	TLM43	650	285 + 300 + 50	420 + 150 + 90	325 + 325	1100
III	TLMBRL38	750	320 + 310 + 105	750	350 + 180 + 160 + 60	1100
IV	TLMBRL26A	750	320 + 310 + 105	750	350 + 200 + 180	1100
V	TLMBRL15	400	220 + 90	280 + 100	210 + 190	1100
VI	TLMBRL169	620	300 + 260 + 60	400 + 120 + 85	320 + 300	1100
VII	TLM1	880	385 + 365	320 + 230 + 180 + 150	365 + 155	1100
VIII	TLM135	790	300 + 275 + 110 + 90	690 + 100	390 + 225 + 150	1100

All values for the 5.8S-ITS PCR, 26S PCR and restriction fragments are given in bp.
Abbreviations: R.P., restriction profile; n.c., not cut.
[a] Restriction enzymes *Mse*I and *Apa*I did not produce any cut fragment.
[b] According to BlastN search of D1/D2 26S rRNA gene sequences in NCBI database.
[c] The 5.8S-ITS gene was also digested with *Dde*I endonuclease confirming the restriction profile reported by Esteve-Zarzoso et al. (1999).

polymorphism (RFLP) analysis of the region spanning the internal transcribed spacers (ITS1 and ITS2) and the 5.8S rRNA gene as reported by Esteve-Zarzoso et al. (1999).

Five isolates representative of each group were subjected to additional enzymatic restriction targeting the 26 rRNA gene, as reported by Settanni et al. (2012). One isolate per group was further processed by sequencing the D1/D2 region of the 26S rRNA gene to confirm the preliminary identification obtained by restriction fragment length polymorphism analysis. The D1/D2 region was amplified with the primers NL1 and NL4 (O'Donnell, 1993). Polymerase chain reaction (PCR) products were visualised as described by Settanni et al. (2012). DNA sequencing reactions were performed at Primm Biotech S.r.l. (Milan, Italy). The identities of the sequences were determined by BlastN search against the NCBI non-redundant sequence database located at http://www.ncbi.nlm.nih.gov.

2.4. Strain typing of S. cerevisiae isolates

Intraspecific characterisation of isolates belonging to *S. cerevisiae* species was carried out by employing two techniques: interdelta analysis with the primers delta12 and delta21 (Legras and Karst, 2003) and microsatellite multiplex PCR based on the analysis of polymorphic microsatellite loci, i.e., SC8132X, YOR267C and SCPTSY7 (Vaudano and Garcia-Moruno, 2008). The PCR products were analysed on 2% agarose gels (w/v) in 1 × Tris/borate/EDTA buffer and visualised as reported by Settanni et al. (2012).

2.5. Chemical analyses

2.5.1. Conventional parameters

The pH, alcoholic content, total titratable acidity, volatile acidity and total and free sulphur dioxide (SO_2) were determined in accordance with the official methods described in the Commission Regulation 2776/90 (1990).

The amount of reducing sugars was determined following the procedures described by Lane and Eynon (1923). The total phenol content was determined according to the Folin-Ciocalteu procedure (Narr Ben et al., 1996). Free anthocyanins were measured by the pH-dependent change in the colour, as proposed by Ribéreau-Gayon and Stonestreet (1965).

The analysis of glycerol was carried out as follows: 1 mL of wine was concentrated in a rotary evaporator at 80 ± 1 °C and the dried residue was dissolved in 2 mL of ethanol. The obtained solution was filtered and 1 μL was subjected to gas-chromatograph (GC) analysis on the GC1000 (Dani Instrument, Cologno Monzese, MI, Italy) equipped with a programmed temperature vaporizer, flame ionisation detector and capillary column (30 m, 250 mm i.d., 0.20 μm film thickness) (Rt2330, Restek Corporation, Bellefonte, PA, USA). Glycerol was identified by comparing the retention time with that of pure glycerol (Sigma Aldrich, St. Louis, MO) and quantification was performed using the external standard method, i.e., calibration curve was constructed with different concentrations (1, 2, 5 and 10 mg/L).

2.5.2. Organic acids

Organic acids were analysed as suggested by Palma and Barroso (2002), with some modifications. Samples were sonicated (Bandelin, Sonorex, Berlin, German) for 10 min at 40 ± 1 °C and 1-mL aliquots were diluted (1:10) with milliQ H_2O, centrifuged at 10,000 rpm for 10 min and filtered (Millipore 0.45 filters). Chromatographic analysis of the samples was carried out as reported by Pereira et al. (2010).

2.5.3. Phenolic and antioxidant activity

Briefly, 10 mL of wine, acidified with ultrapure HCl to pH 2.0, was extracted three times with 20 mL of ethyl ether. The organic phase was concentrated on a rotary evaporator at 30 ± 1 °C and the residue was dissolved in 10 mL of a water/methanol mixture (1:1). The analysis was carried out by high-performance liquid chromatography with diode-array detection, as described by Sakakibara et al. (2003).

Quantification was performed using the external standard method by constructing calibration curves. For this, (+)-catechin, (−)-epicatechin, vanillic acid, gallic acid, caffeic acid, *p*-coumaric acid, syringic acid, chlorogenic acid, sinapic acid, ellagic acid, resveratrol and malvidin chloride were purchased from Sigma-Aldrich.

The analysis of the antioxidant activity was carried out according to the technique reported by Brand-Williams et al. (1995). The antioxidant activity was expressed as mM trolox-equivalents, a vitamin E analogue.

2.5.4. VOCs

Briefly, 10 mL of wine, spiked with 37.5 μg of 1-decanol as internal standard (I.S.), was mixed with 1 mL of a 26% NaCl solution and subsequently extracted with 1 mL of dichloromethane. Samples were stirred for 5 min and then centrifuged at 5 °C for 5 min at 5000 rpm. The VOC analyses were carried out on an Agilent GC 6890 gas chromatograph coupled to an Agilent 5973 mass-selective detector. The GC–mass spectrometry (MS) conditions suggested by Boch-Fusté et al. (2007) were employed. VOC identification was achieved by comparing the mass spectra and GC retention times with those of the pure standard compounds that were available and the data system library of the GC–MS equipment (NIST 02 and WILEY 275) with a similar index (i.e., >90%). The concentrations of the compounds were estimated

Size of restriction fragments[a]	Species (% identity)[b]	Acc. no.	Distribution (no. of isolates)		
HinfI			Must	Alcoholic fermentation	Post-fermentation maceration
n.c.	*Aureobasidium pullulans* (99)	KF263940	(28)		
445 + 390 + 180 + 50	*Debaryomyces carsonii* (99)	JX456534			60 d (41)
n.c.	*Hanseniaspora guilliermondii*[c] (99)	KF263937		3 d (31), 8 d (24)	
n.c.	*Hanseniaspora uvarum*[c] (99)	KF263939		3 d (22), 8 d (27)	
n.c.	*Metschnikowia pulcherrima* (99)	KF263936	(37)	3 d (25)	
n.c.	*Pichia guilliermondii* (99)	KF263938	(29)	3 d (38)	
500 + 220 + 180	*Saccharomyces cerevisiae* (99)	JX456533		3 d (35), 8 d (32)	13 d (41), 20 d (80), 40d (97), 50 d (77), 60 d (44), 70 d (27), 90 d (23)
445 + 225 + 190 + 125 + 60 + 50	*Zygosaccharomyces bisporus* (99)	JX456535			60 d (37)

by comparing their peak areas with those of the respective I.S. with known concentration.

2.6. Sensory evaluation

The sensory profiles of the experimental wines characterised by different times of maceration were evaluated using a descriptive method (UNI 10957, 2003). Ten judges were trained in a few preliminary sessions, using different samples of commercial wines obtained from the Aglianico cultivar by a post-fermentation maceration of about 12 15 d, in order to develop a common vocabulary for the description of the sensory attributes of wine samples and to familiarise them with scales and procedures. The standards used to define descriptors were chosen according to Noble et al. (1987).

On the basis of the frequency of citation (>60%), 16 descriptors were included in the analysis: colour intensity, odour intensity, odour complexity, off-odours, fresh fruits, dried fruits, flowers, aromatic herbs, spices (odour), sweet, hot (tactile in mouth), acid, astringent, bitter, taste complexity, and off-flavour (taste). The wine samples were randomly evaluated by assigning a score between 1.00 (absence of sensation) and 9.00 (extremely intense) in individual booths under incandescent white light.

2.7. Statistical analysis

All determinations and experiments were performed in triplicate, and the results presented are the average value of three determinations. Using data from chemical analyses, one way analysis of variance (ANOVA) was performed to compare the long-macerated wines. Statistical significance was attributed to P values of <0.05. The post-hoc Tukey test was applied for pairwise comparison. In addition, a principal component analysis (PCA) was carried out. Statistical analysis was performed using XLSTAT 2006, version 2006.6 (Addinsoft, Paris, France). The resulting scores from the sensory analysis were averaged and compared. The ANOVA test (STATISTICA software, StatSoft Inc., Tulsa, OK, USA) was applied to identify significant differences among the wine attributes.

3. Results

3.1. Microbiological analysis

The viable counts of the microbial groups investigated in this study are reported in Fig. 1. The level in grape must was 5.75 Log CFU/mL. The highest TY concentration was reached at beginning of alcoholic fermentation (day 3) and decreased to 7 Log CFU/mL at the end of the tumultuous phase of alcoholic fermentation (day 8). During post-fermentation maceration, the TY concentration remained almost constant at approximately 7 Log CFU/mL until day 40. Subsequently, it decreased, reaching levels between 4.79 Log CFU/mL (day 50) and 6.18 Log CFU/mL (day 90).

The LAB populations reached a detectable value at the end of the tumultuous phase of alcoholic fermentation. Their concentration decreased by day 20 of post-fermentation maceration, both on MRS agar and MLO, and was estimated at about 5 Log CFU/mL at the end of the experimental process. Furthermore, no consistent difference was found between total and acidophilic (presumptive *Oenococci*) LAB during the entire period of observation. *Dekkera*/*Brettanomyces* spp. as well as AAB were not detected, even after filtration of the samples.

3.2. Isolation, identification and distribution of yeasts

A total of 795 colonies were isolated from WL, purified to homogeneity, and separated based on the appearance of colony morphology. All colonies were subjected to molecular identification. After restriction analysis of the 5.8S-ITS region and 26S rRNA gene, the isolates were clustered into eight groups (Table 1). *Hanseniaspora guilliermondii* (group III), *Hanseniaspora uvarum* (group IV), *Metschnikowia pulcherrima* (group V), *S. cerevisiae* (group VII) and *Zygosaccharomyces bisporus* (group VIII) were directly identified by comparison of the restriction bands with those available in the literature (Settanni et al., 2012; Esteve-Zarzoso et al., 1999). The identification of groups I, II and IV was concluded by sequencing of the D1/D2 domain of the 26S rRNA gene, which allocated these isolates into the *Aureobasidium pullulans*, *D. carsonii* and *Pichia guilliermondii* species, respectively. The species *A. pullulans* was detected only in the must; on the other hand, *M. pulcherrima* and *P. guilliermondii* were found both in the must and in samples collected on day 3 of alcoholic fermentation. *H. guilliermondii* and *H. uvarum* as well as *S. cerevisiae* were the dominant species during the tumultuous phase of alcoholic fermentation. During the entire process of post-fermentation maceration, *S. cerevisiae* was the species found at the highest concentrations, whereas *D. carsonii* and *Z. bisporus* were detected only on day 60, at concentrations of 5.31 and 5.79 Log CFU/mL, respectively.

3.3. Typing and distribution of S. cerevisiae strains

The 456 isolates belonging to the species *S. cerevisiae* were further genetically characterised. The interdelta analysis was able to separate the isolates into nine groups, whereas microsatellite multiplex PCR

recognised seven different groups (data not shown), showing a lower discriminatory power than the former technique.

The distribution of the different strains of *S. cerevisiae* during the experimental vinification is reported in Fig. 2. No *S. cerevisiae* strains were detected in the must; on the other hand, during the tumultuous phase of alcoholic fermentation, only the deltaA profile was detected. This pattern and the deltaC and deltaD profiles were mainly isolated during the first days of maceration (13–20 d), in the intermediate phase (40–60 d), and at the end of the process (90 d), respectively. The deltaA profile corresponding to *S. cerevisiae* NF66 (inoculated starter) was detected from day 3 of fermentation until the end of maceration. In terms of diversity of *S. cerevisiae*, the highest numbers of different strains at high concentrations were found between days 40 and 50 of maceration.

3.4. Chemical analyses

The conventional parameters and organic acid contents of the wine samples during vinification are reported in Table 2.

Most of the reducing sugars were converted to ethanol due to the metabolic activities of yeast during the tumultuous phase of alcoholic fermentation; they were not detectable on day 70 of post-fermentation maceration. The alcohol content was 12.51% (v/v) at the end of the tumultuous phase of fermentation and was 13.67% (v/v) at the end of the maceration process.

Furthermore, pH and volatile acidity contents increased during the monitored vinifications, whereas an opposite behaviour was observed for total titratable acidity. The total phenol content varied with the time of contact of grape skins and seeds with the wine. During fermentation, it increased to 1167.42 mg/L (gallic acid) at the end of fermentation, but from days 13 to 40 of post-fermentation maceration, its concentration increased by about 38% and remained almost constant until the end of the process.

The highest concentration of total anthocyanins (241.48 mg/L) was noted on day 50 of post-fermentation maceration, but the value estimated at the end of the process was almost 100 mg/L lower; however, total and free SO_2 showed constant values during vinification.

The amount of glycerol increased consistently during the tumultuous phase of alcoholic fermentation (Table 2). Its concentration increased by less than 2.3 g/L during the post-maceration period, reaching 13.38 g/L at the end of the process.

The concentration of tartaric acid decreased to approximately 2 g/L in the intermediate phase of post-fermentation maceration. Malic acid showed a decreasing trend during the entire experimental period. The lactic acid content increased to 2.65 g/L at the end of the tumultuous phase of alcoholic fermentation; thereafter, its concentration did not vary greatly ($P > 0.05$) between the beginning and the end of post-fermentation maceration. However, the lowest values were observed between days 40 and 50 of maceration.

The phenolic compounds identified and quantified during experimental vinification are reported in Table 3. The main compounds belonging to this family were benzoic and cinnamic acids and flavan-3-ols, which, together, represented more than 90% of the phenolic fraction of the must and wine at any time of collection. In general, the phenolic concentration increased with increasing maceration time, reaching a maximum value (854.9 mg/L) on day 90 of post-fermentation maceration. However, the rate of increase was higher during the first 40 d of maceration: a decrease in the rate was noted between day 40 (806.60 mg/L) and day 50 (653.40 mg/L), after which it increased again. In particular, the concentration of hydroxybenzoic and hydroxycinnamic acids and phenols reached a maximum on day 40 showing values of 283.40, 255.00 and 8.00 mg/L, respectively.

Among the benzoic acids, gallic acid was the most prevalent acid starting from day 40 of maceration. However, the highest concentration was measured for syringic acid on days 13 and 20 and its concentration (range: 35.82–49.04 mg/L) did not vary greatly throughout the experiment. Except on day 50, caffeoylquinic acid was the cinnamic acid with the highest concentration (>150 mg/L). The main flavan-3-ol identified was (+)-catechin, whose concentration showed an irregular trend during the winemaking process. The same behaviour was observed for (−)-epicatechin. In particular, at day 8 of alcoholic fermentation, the ratio between (+)-catechin and (−)-epicatechin was 3.57; it decreased to 2.82 on day 20 of post-fermentation maceration and, thereafter, it reached the highest value (3.58) on day 40 and the lowest value (2.0) on day 90 of maceration.

Among the phenolic compounds present at concentrations lower than those of benzoic acids, cinnamic acids and catechins, stilbenes were represented by α-viniferin (a trimer of resveratrol) and *trans*-resveratrol. In addition, it is worth noting that among the flavonols, rutin and quercetin were detected.

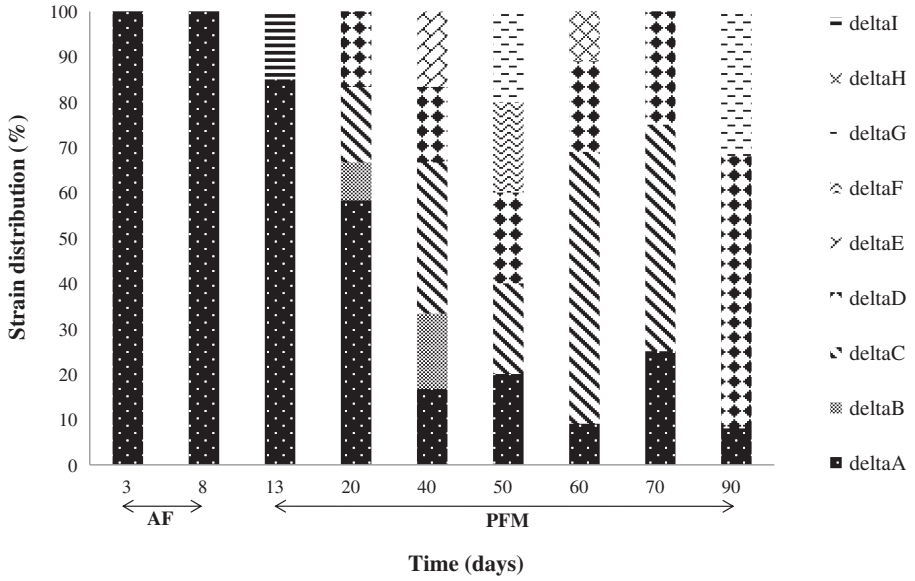

Fig. 2. Distribution of *S. cerevisiae* strains during experimental vinification of "Aglianico di Taurasi" wine. Abbreviations: AF, alcoholic fermentation; PFM, post-fermentation maceration. The nine strains are coded as deltaA, deltaB, deltaC, deltaD, deltaE, deltaF, deltaG, deltaH and deltaI.

Table 2
Chemical parameters during extended maceration of "Aglianico di Taurasi" wine.

	Conventional parameters										Organic acids (g/L)			
	pH	Reducing sugars (g/L)	Alcohol (% v/v)	TTA	VA	TP	TA	Glycerol	Total SO_2	Free SO_2	Tartaric acid	Acetic acid	Malic acid	Lactic acid
Must	$3.25^{g} \pm 0.03$	$236.14^{a} \pm 2.11$	n.d.^{h}	$8.86^{a} \pm 0.15$	$0.17^{f} \pm 0.04$	$349.22^{j} \pm 24.21$	$150.44^{h} \pm 9.76$	n.d.^{j}	n.d.^{j}	n.d.^{j}	$2.78^{d} \pm 0.07$	n.d.^{g}	$2.89^{d} \pm 0.27$	n.d.
AF-day 3	$3.37^{e} \pm 0.05$	$152.87^{b} \pm 3.12$	$3.84^{g} \pm 0.05$	$8.66^{b} \pm 0.21$	$0.49^{a} \pm 0.02$	$987.84^{i} \pm 37.62$	$191.07^{d} \pm 16.73$	$4.24^{i} \pm 0.50$	$49.08^{a} \pm 0.24$	$26.08^{a} \pm 0.32$	$3.22^{a} \pm 0.07$	$0.33^{d,e} \pm 0.09$	$3.11^{b,c} \pm 0.11$	$0.18^{h} \pm 0.10$
AF-day 8	$3.30^{f} \pm 0.07$	$10.60^{e} \pm 2.07$	$12.51^{f} \pm 0.04$	$8.17^{d} \pm 0.09$	$0.37^{d} \pm 0.07$	$1167.42^{b} \pm 38.93$	$201.67^{b} \pm 11.16$	$9.13^{h} \pm 0.33$	$44.05^{b} \pm 0.31$	$25.05^{b} \pm 0.11$	$3.13^{b} \pm 0.07$	$0.31^{e} \pm 0.07$	$3.09^{c} \pm 0.10$	$2.65^{g} \pm 0.11$
PFM-day 13	$3.29^{f} \pm 0.01$	$5.98^{d} \pm 1.47$	$12.79^{f} \pm 0.04$	$8.39^{c} \pm 0.35$	$0.30^{e} \pm 0.02$	$1326.78^{g} \pm 53.46$	$189.25^{c} \pm 13.76$	$11.15^{g} \pm 0.72$	$43.02^{c} \pm 0.62$	$24.63^{c} \pm 0.21$	$3.01^{c} \pm 0.07$	$0.27^{f} \pm 0.03$	$3.12^{b} \pm 0.23$	$2.91^{e} \pm 0.21$
PFM-day 20	$3.45^{d} \pm 0.00$	$2.96^{e} \pm 2.21$	$12.97^{e,f} \pm 0.11$	$8.15^{d} \pm 0.37$	$0.39^{c,d} \pm 0.00$	$1592.86^{f} \pm 31.69$	$195.83^{c} \pm 16.98$	$12.89^{b} \pm 0.47$	$41.02^{e} \pm 0.40$	$21.47^{e} \pm 0.19$	$2.72^{e} \pm 0.47$	$0.33^{d,e} \pm 0.11$	$2.75^{e} \pm 0.17$	$2.87^{b} \pm 0.30$
PFM-day 40	$3.60^{b} \pm 0.10$	$2.15^{g} \pm 2.13$	$13.25^{b} \pm 0.07$	$6.95^{h} \pm 0.05$	$0.40^{c} \pm 0.02$	$1825.91^{e} \pm 21.60$	$183.32^{g} \pm 14.78$	$12.11^{f} \pm 0.55$	$38.55^{i} \pm 0.33$	$20.87^{g} \pm 0.44$	$2.03^{h} \pm 0.55$	$0.35^{c,d} \pm 0.04$	$2.21^{a} \pm 0.11$	$2.79^{c} \pm 0.29$
PFM-day 50	$3.56^{c} \pm 0.00$	$2.07^{h} \pm 1.65$	$13.41^{d} \pm 0.35$	$7.00^{g} \pm 0.18$	$0.40^{c} \pm 0.02$	$1838.02^{c} \pm 47.83$	$241.48^{a} \pm 12.18$	$12.35^{c} \pm 0.52$	$40.30^{g} \pm 0.19$	$22.63^{d} \pm 0.12$	$2.02^{h,i} \pm 0.52$	$0.31^{e} \pm 0.10$	$2.06^{f} \pm 0.09$	$2.56^{d} \pm 0.11$
PFM-day 60	$3.57^{c} \pm 0.00$	$1.98^{i} \pm 0.97$	$13.59^{c} \pm 0.21$	$7.19^{f} \pm 0.18$	$0.43^{b} \pm 0.06$	$1861.62^{b} \pm 25.97$	$188.81^{c} \pm 15.97$	$12.30^{d} \pm 0.32$	$42.12^{d} \pm 0.27$	$21.11^{f} \pm 0.26$	$2.00^{g,h} \pm 0.32$	$0.38^{b} \pm 0.12$	$2.02^{g} \pm 0.10$	$2.58^{f} \pm 0.09$
PFM-day 70	$3.57^{a} \pm 0.01$	n.d.^{f}	$13.62^{b} \pm 0.17$	$6.94^{h} \pm 0.23$	$0.47^{a} \pm 0.04$	$1915.01^{a} \pm 28.08$	$145.67^{f} \pm 11.08$	$12.17^{e} \pm 0.27$	$39.06^{h} \pm 0.18$	$20.02^{i} \pm 0.61$	$2.11^{i} \pm 0.27$	$0.41^{a} \pm 0.11$	$2.05^{f} \pm 0.21$	$2.56^{f} \pm 0.11$
PFM-day 90	$3.63^{a} \pm 0.03$	n.d.^{f}	$13.67^{b} \pm 0.28$	$7.31^{e} \pm 0.20$	$0.41^{b,c} \pm 0.06$	$1837.47^{d} \pm 32.78$	$149.60^{f} \pm 10.57$	$13.38^{d} \pm 0.78$	$40.78^{f} \pm 0.50$	$20.09^{h} \pm 0.59$	$2.25^{f} \pm 0.78$	$0.37^{b} \pm 0.09$	$1.59^{h} \pm 0.18$	$3.04^{a} \pm 0.12$

AF, alcoholic fermentation; PFM, post-fermentation maceration; TTA, total titratable acidity (tartaric acid g/L); VA, volatile acidity (acetic acid g/L); TP, total phenols (gallic acid mg/L); TA, total anthocyanins (malvidin chloride mg/L); glycerol (g/L); total SO_2 and free SO_2 (mg/L); n.d., not determined (values < detection limit).
a–j: Different letters indicate significant differences between maceration time for the same sample for $P \leq 0.05$.

The VOC family (Table 4) is composed of alcohols, esters, carbonyl compounds, acids, lactones, phenols, hydrocarbons and other compounds. Alcohols were not detected in the must but found at concentrations of approximately 1.4 g/L on day 8 of alcoholic fermentation and about 1.7 g/L on day 90 of post-fermentation maceration. The main compounds within this class were 1-butanol, 3-methyl and phenylethyl alcohol, which constituted more than 98% of total alcohols. The ester concentration was 18.20 mg/L on day 8 of fermentation and reached the highest value (52.57 mg/L) on day 90 of maceration. Among the most frequently identified esters, the contents of 1-butanol, 3-methyl-,acetate and hexanoic acid, ethyl ester decreased during maceration, whereas an opposite behaviour was observed for butanedioic acid, diethyl ester and propanoic acid, 2-hydroxy-,ethyl ester. The main compounds among aldehydes and ketones were nonanal and 5-phenyl-2-pentanone, respectively; the concentrations of both compounds decreased during the experimental period. Methyl 4-hydroxybutanoate was the major acid found. Its concentration increased to a maximum level (44.68 mg/L) on day 40 of maceration and remained almost constant until day 90. No significant difference was observed with regard to the concentration of γ-butyrolactone between day 13 and day 90 (8.28 and 8.10 mg/L, respectively). The phenol class was mostly represented by 1H-indole-3-ethanol.

The antioxidant activity (data not shown) in the must was 2.56 mM trolox equiv. and increased to 7.98 mM trolox equiv. on day 8 of fermentation. The highest antioxidant activity (around 9.48 mM trolox equiv.) of wine was observed between day 40 and day 60 of maceration. These values were significantly different from that measured on day 13.

3.5. PCA of VOCs

The F1 and F2 components, selected from the PCA analysis, explain 72.09 and 17.15% of total variance, respectively, as illustrated in Fig. 3.

The main descriptors contributing to the F1 component were: alcohols, lactones, fatty acids, phenols and hydrocarbons of which the loading value were 0.869, 0.823, 0.720, 0.708 and 0.706, respectively. On the other hand, the F2 component was mainly characterised by other compounds, esters, hydrocarbons and fatty acids; their respective loading values were 0.580, 0.281, 0.166 and 0.148.

Except for the must and the samples taken after day 8 (AF-Day8) and day 20 (PFM-Day20) of post-fermentation maceration, the wines were located in the quadrant showing positive values of the F1 component. In particular, the samples macerated for 50 days (PFM-Day50) and 70 days (PFM-Day70) were located in the same area showing similar values of F1 components. On the other hand, among the macerated wines, the samples taken after 20 days (PFM-Day20) and 90 days (PFM-Day90) of maceration were furthest separated according to their different values in terms of F1 components.

3.6. Sensory analysis

The wines obtained by using different periods of post-fermentation maceration were evaluated by sensory analysis and the results are reported in Table 5. Samples subjected to 40, 50 and 90 d of maceration differed significantly ($P < 0.05$) from the other samples with regard to the majority of odour and taste descriptors. In particular, the highest values of odour intensity as well as odour and taste complexity were displayed by the 40, 50 and 90 day samples. The wines on days 40 and 90 showed also the lowest values of acid, astringent and bitter descriptors (taste). No off-odours and off-flavours were detected in all samples analysed.

4. Discussion

The aim of the present study was to evaluate the influence of post-fermentation maceration, extended to 90 days, on the microbial and chemical composition of red wine. Microbiological results evidenced

Table 3
Concentration (mg/L) of phenols during experimental vinification of "Aglianico di Taurasi" wine.

	Must	Alcoholic fermentation	Post-fermentation maceration				
		Day-8	Day-13	Day-20	Day-50	Day-70	Day-90
Σ hydroxybenzoic acids	12.93^{i}	78.27^{i}	144.55^{g}	172.50^{g}	203.30^{e}	266.40^{c}	282.30^{b}
Gallic acid	3.22^{i} ± 0.56	7.59^{h} ± 1.06	26.25^{g} ± 0.93	34.92^{g} ± 0.99	79.77^{e} ±2.82	117.80^{c} ± 3.17	126.80^{b} ± 3.77
Protocatechuic acid	n.d.^{i}	4.33^{h} ± 0.14	5.90^{g} ± 0.21	7.10^{d} ± 0.20	6.56^{g} ±0.23	7.33^{c} ± 0.20	7.49^{b} ± 0.22
p-Coumaroylquinic acid	9.71^{i} ± 1.34	30.77^{h} ± 1.67	55.06^{e} ± 1.95	63.13^{b} ± 1.79	50.11^{f} ± 1.77	55.4d^{a} ± 1.49	57.06^{c} ± 1.69
Vanillic acid	n.d.^{i}	4.25^{h} ± 0.77	9.21^{e} ± 0.33	10.68^{a} ± 0.30	8.71^{f} ± 0.31	9.34^{d} ± 0.25	9.91^{c} ± 0.29
Syringic acid	n.d.^{j}	31.33^{h} ± 1.07	42.08^{d} ± 1.49	49.04^{a} ± 1.39	37.10^{f} ± 1.31	44.78^{c} ± 1.20	41.06^{e} ± 1.22
Acylated syringic acid	n.d.^{h}	n.d.^{h}	6.05^{g} ± 0.21	7.58^{f} ± 0.21	21.04^{e} ± 0.74	31.61^{b} ± 0.85	40.01^{a} ± 1.19
Σ hydroxycinnamic acids	80.39^{i}	130.01^{h}	179.09^{e}	202.60^{c}	175.30^{g}	212.50^{b}	213.60^{d}
Caffeoylquinic acid	80.39^{i} ± 2.78	120.7^{h} ± 3,45	157.91^{a} ± 5.58	176.5^{b} ± 4.99	146.40^{d} ± 5.18	177.5^{b} ± 4.77	168.90^{e} ± 5.02
Hydroxycinnamic acid	n.d.^{g}	n.d.^{g}	10.45^{c} ± 0.37	12.00^{a} ± 0.34	8.23^{f} ± 0.29	9.69^{e} ± 0.26	9.76^{d} ± 0.29
Caffeic acid	n.d.^{i}	7.11^{g} ± 0.18	6.18^{h} ± 0.22	6.25^{g} ± 0.18	7.97^{e} ± 0.28	9.93^{c} ± 0.27	13.1^{a} ± 0.39
p-Coumaric acid	n.d.^{h}	2.29^{g} ± 0.22	4.55^{f} ± 0.16	7.87^{e} ± 0.22	12.69^{d} ± 0.45	15.41^{b} ± 0.41	21.84^{a} ± 0.65
Σ flavones	n.d.^{i}	4.55^{e}	8.09^{b}	8.53^{a}	3.16^{h}	4.74^{d}	3.92^{f}
Luteolin 7-*O*-glucoside	n.d.^{i}	4.55^{e} ± 0.15	8.09^{b} ± 0.27	8.53^{a} ± 0.24	3.16^{h} ± 0.11	4.74^{d} ± 0.13	3.92^{f} ± 0.12
Σ flavonols	n.d.^{g}	2.01^{f}	4.94^{d}	n.d.^{g}	2.81^{e}	n.d.^{g}	6.93^{b}
Rutin	n.d.^{f}	2.01^{e} ± 0.23	4.94^{c} ± 0.17	n.d.^{f}	2.81^{d} ± 0.10	n.d.^{f}	4.91^{c} ± 0.15
Quercetin	n.d.^{b}	n.d.^{b}	n.d.^{b}	n.d.^{b}	n.d.^{b}	n.d.^{b}	2.02^{a} ± 0.06
Σ flavanones	n.d.^{g}	3.48^{g}	5.27^{d}	6.86^{a}	5.43^{c}	6.18^{b}	4.23^{f}
Narigenin	n.d.^{h}	3.48^{g} ± 0.45	5.27^{d} ± 0.19	6.86^{a} ± 0.19	5.43^{c} ± 0.19	6.18^{b} ± 0.17	4.23^{f} ± 0.13
Σ flavan-3-ols	16.55^{i}	106.31^{h}	148.44^{g}	155.00^{f}	240.00^{c}	190.10^{e}	310.50^{a}
(−)-Gallocatechin	n.d.^{f}	n.d.^{f}	2.42^{a} ± 0.09	0.93^{c} ± 0.03	0.84^{d} ± 0.03	0.73^{e} ± 0.02	1.08^{b} ± 0.03
(−)-Epigallocatechin	10.01^{i} ± 0.49	52.83^{e} ± 1.34	53.89^{d} ± 1.90	59.19^{a} ± 1.67	47.26^{h} ± 1.67	51.08^{f} ± 1.37	54.18^{c} ± 1.61
(+)-Catechin	6.54^{i} ± 1.67	37.49^{h} ± 2.07	58.14^{g} ± 2.06	59.52^{f} ± 1.68	119.1^{c} ±4.21	93.95^{f} ± 2.52	156.90^{a} ± 4.66
(−)-Epicatechin	n.d.^{i}	10.21^{h} ± 0.88	20.57^{g} ± 0.73	25.19^{f} ± 0.71	54.16^{c} ± 1.91	29.87^{e} ± 0.80	78.30^{a} ± 2.32
Epicatechin gallate	n.d.^{h}	n.d.^{h}	2.92^{g} ± 0.10	3.34^{f} ± 0.09	4.42^{e} ± 0.16	4.78^{c} ± 0.13	6.89^{b} ± 0.20
Catechin gallate	n.d.^{i}	5.78^{h} ± 0.32	10.50^{e} ± 0.37	6.78^{g} ± 0.19	14.17^{a} ± 0.50	9.71^{f} ± 0.26	13.17^{c} ± 0.39
Σ phenols	n.d.^{i}	2.44^{h}	5.78^{e}	3.21^{g}	5.72^{f}	6.61^{c}	6.93^{b}
Pyrogallol	n.d.^{i}	2.44^{h} ± 0.19	5.78^{e} ± 0.20	3.21^{g} ± 0.09	5.72^{f} ± 0.20	6.61^{c} ± 0.18	6.93^{b} ±0.21
Σ stilbenes	n.d.^{i}	2.21^{h}	6.15^{g}	9.92^{e}	9.42^{f}	11.55^{b}	15.78a
α-Viniferin	n.d.^{i}	1.27^{h} ± 0.11	3.47^{g} ± 0.12	5.48^{c} ± 0.15	4.05^{f} ± 0.14	5.31^{d} ± 0.14	6.30^{a} ± 0.19
trans-Resveratrol	n.d.^{i}	0.94^{h} ± 0.23	2.68^{g} ± 0.09	4.44^{f} ± 0.13	5.37^{d} ± 0.19	6.24^{c} ± 0.17	9.48^{a} ± 0.28
Σ lignans	n.d.^{h}	5.87^{g}	8.94^{c}	7.56^{f}	8.32^{d}	12.14^{b}	10.62^{a}
Unknown	n.d.^{h}	3.67^{e} ± 0.12	4.73^{c} ± 0.17	4.70^{c} ± 0.13	3.47^{f} ± 0.12	6.25^{a} ± 0.17	3.94^{d} ± 0.12
Flavolignan	n.d.^{h}	2.21^{g} ± 0.10	4.21^{d} ± 0.15	2.86^{f} ± 0.08	4.85^{c} ± 0.17	5.89^{b} ± 0.16	6.68^{a} ± 0.20
Total	109.87^{i}	329.75^{h}	505.50^{g}	566.10^{f}	653.40^{e}	710.20^{c}	854.90^{a}

n.d.: Not detected (value under detection limit of 0.5 mg/L).
a–i: Different letters indicate significant differences between maceration time for the same sample for $P \leq 0.05$.

substantial concentrations of both yeast and LAB populations during the entire period of post-fermentation maceration. Furthermore, the changes in their concentrations, during the experimental process suggested the presence of yeasts and LAB that were not only alive but also metabolically active. To our knowledge, no work has been carried out on the evaluation of the concentrations of yeasts and LAB as well as their analysis at the species and/or strain level during prolonged post-fermentation maceration.

In this work, during the entire maceration process, *S. cerevisiae* was the main species found; in particular, except on day 60 when it was detected at the same level as *D. carsonii* and *Z. bisporus*, it dominated the yeast population. *D. carsonii*, a yeast species that is not commonly associated with the wine environment, has been reported to possess high β-glucosidase activity (Hernàndez-Orte et al., 2008), contributing to the characteristic aroma of grapevine varieties (Zott et al., 2008). *Z. bisporus* is not reported to be a relevant spoilage yeast species for wines (Loureiro and Malfeito-Ferreira, 2003), although its presence has been associated with the production of off-flavours in sherry wine (Neuser et al., 2000).

However, very low species diversity was observed, which could be explained by the stressing conditions (high ethanol concentration, low pH and scarcity of nutrients) that characterise the process and result in a strong selection. On the other hand, these stressing conditions did not negatively affect the yeast diversity at the strain level of the *S. cerevisiae* community. In fact, excluding the starter, eight different indigenous strains were detected during maceration and found at high levels. Our results confirmed previous observations that the cellar environment is a source of indigenous strains (Le Jeune et al., 2006; Guzzon et al., 2011) that, once adapted to winemaking process, could also be dominant under stressing conditions such as those that are characteristic of prolonged maceration.

Furthermore, our work described for the first time the effects of the activities of yeasts and LAB on wine composition during post-fermentation maceration extended to 90 days. In particular, yeast activities were clearly observed, i.e., lactic acid was degraded and malic acid was converted to ethanol (Redzepovic et al., 2003) during the first phase of maceration (from days 13 to 50). In addition, glycerol production could be associated with the microbial metabolism due to glycero-pyruvic fermentation observed during the increase in yeast at the end of the maceration process (from days 70 to 90). As observed by several authors (Gardner et al., 1993; Hernandez-Cortes et al., 2010), glycerol could be produced by yeasts even if their concentration in wine is low, at different phases of the winemaking process and under stressing conditions.

During maceration, although at low concentrations, the influence of LAB on the chemical composition of the wines was clearly shown. The LAB activities started with the production of lactic acid by homolactic fermentation on day 13 of maceration and were terminated with the degradation of malic acid by malolactic fermentation between days 70 and 90. Furthermore, in this context, several studies (Alexandrea et al., 2004; Capucho and San Romao, 1994; Schutz and Radler, 1974) showed the capability of LAB to carry out fermentation even at very low concentrations as well as under stressing conditions and during different phases of the winemaking process.

The production of glycerol and lactic acid is a desired phenomenon with regard to sensory characteristics of red wines because they

Table 4
Concentration of volatile organic compounds (mg/L) during experimental vinification of "Aglianico di Taurasi" wine.

Compounds	Descriptors	Must	Alcoholic fermentation	Post-fermentation maceration				
			Day-8	Day-13	Day-20	Day-50	Day-70	Day-90
Σ alcohols		n.d.^{i}	1478.97^{h}	1918.34	1563.42	1687.40	1598.57	1736.72
1-Butanol, 3-methyl-	Burnt, alcohol	n.d.^{i}	865.89^{f} ± 1.23	930.90^{b,c} ± 7.07	855.01^{e} ± 0.71	933.04 ± 3.54	856.27^{d,e} ± 0.71	867.92^{d} ± 1.41
1-Butanol, 2-ethyl-	Burnt, alcohol	n.d.^{c}	n.d.^{c}	0.78^{a} ± 0.00	0.34^{b} ± 0.01	n.d.^{c}	n.d.^{c}	n.d.^{c}
1,2-Propanediol	Ripe fruit, alcohol	n.d.^{b}	n.d.^{b}	n.d.^{b}	n.d.^{b}	n.d.^{b}	n.d.^{b}	n.d.^{b}
2,3-Butanediol	Fruity	n.d.^{h}	6.09^{g} ± 0.06	11.33^{b} ± 0.01	8.91^{d} ± 0.01	24.68^{a} ± 0.01	7.40^{f} ± 0.01	9.05^{c} ± 0.01
2-Heptanol, 3-methyl-	Humid	n.d.^{h}	1.07^{e} ± 0.04	2.21^{b} ± 0.02	1.38^{d} ± 0.01	5.24^{a} ± 0.01	0.96^{f} ± 0.01	1.54^{c} ± 0.01
3-Buten- 2-ol, 2-methyl-	Herbaceous	n.d.^{b}	n.d.^{b}	n.d.^{b}	1.60^{a} ± 0.01	n.d.^{b}	n.d.^{b}	n.d.^{b}
1-Propanol, 3-ethoxy-	Fruity	n.d.^{h}	0.54^{g} ± 0.03	0.78^{g} ± 0.00	0.90^{f} ± 0.01	0.95^{e} ± 0.01	1.80^{b} ± 0.01	1.95^{a} ± 0.01
1-Pentanol, 3-methyl-	Vinous, herbaceous, cacao	n.d.^{g}	1.11^{f} ± 0.02	1.17^{e} ± 0.00	1.07^{f} ± 0.01	1.17^{e} ± 0.01	2.52^{c} ± 0.01	3.41^{a} ± 0.01
1-Butanol, 2,3-dimethyl-	Bitter, solvent	n.d.^{e}	n.d.^{e}	n.d.^{e}	0.73^{c} ± 0.01	n.d.^{e}	1.08^{a} ± 0.01	0.55^{d} ± 0.01
1-Hexanol	Flower, green, cut grass	n.d.^{g}	4.27^{a} ± 0.00	6.76^{a} ± 0.00	4.68^{e} ± 0.01	4.32^{g} ± 0.01	4.35^{f} ± 0.01	6.00^{b} ± 0.01
5-Nonanol		n.d.^{c}	n.d.^{c}	n.d.^{d}	n.d.^{d}	n.d.^{d}	1.09^{a} ± 0.01	1.07^{b} ± 0.01
2-Heptanol, 4-methyl-	Humid	n.d.^{d}	n.d.^{d}	0.11^{e} ± 0.00	0.00^{f} ± 0.00	0.00^{f} ± 0.00	0.51^{b} ± 0.01	0.56^{a} ± 0.01
Phenylethyl Alcohol	Floral, roses	n.d.^{i}	599.72^{h} ± 4.22	963.64^{a} ± 3.54	688.51^{f} ± 2.12	717.67^{e} ± 0.04	722.16^{e} ± 1.41	843.83^{c} ± 1.41
1,2-Butanediol, 1-phenyl-	Bitter, solvent	n.d.^{e}	0.28^{d} ± 0.02	0.68^{b} ± 0.01	0.29^{e} ± 0.01	0.33^{d} ± 0.01	0.44^{c} ± 0.01	0.83^{a} ± 0.01
Σ esters		7.34^{i}	18.20^{g}	25.44	16.70	26.34	34.20	52.57
1-Butanol, 3-methyl-, acetate	Roses, flowery	n.d.^{i}	4.22^{g} ± 0.01	7.68^{a} ± 0.01	4.45^{e} ± 0.01	4.75^{d} ± 0.01	4.28^{f} ± 0.01	3.76^{g} ± 0.01
1-Butanol, 2-methyl-, acetate	Roses, flowery	n.d.^{e}	0.32^{d} ± 0.00	0.43^{d} ± 0.00	n.d.^{e}	0.46^{c} ± 0.01	0.54^{b} ± 0.01	0.47^{c} ± 0.01
Butanedioic acid, diethyl ester		2.07^{g} ± 0.04	2.09^{g} ± 0.02	3.27^{f} ± 0.01	2.04^{g} ± 0.01	5.43^{e} ± 0.01	12.40^{b} ± 0.01	29.35^{a} ± 0.01
Octanoic acid, ethyl ester	Fruit, sweet, soap, anise	n.d.^{f}	n.d.^{f}	0.94^{b} ± 0.01	n.d.^{f}	0.97^{b} ± 0.01	0.53^{d} ± 0.01	0.41^{e} ± 0.01
Propanoic acid, 2-hydroxy-, ethyl ester		3.44^{i} ± 0.02	7.45^{f} ± 0.02	6.44^{f} ± 0.01	6.06^{g} ± 0.01	9.57^{e} ± 0.01	11.92^{c} ± 0.01	14.61^{b} ± 0.01
Butanoic acid, 3-hydroxy-, ethyl ester,		n.d.^{e}	n.d.^{e}	0.49^{a} ± 0.01	0.22^{e} ± 0.01	0.00^{f} ± 0.00	0.37^{c} ± 0.01	0.42^{b} ± 0.01
Hexanoic acid, ethyl ester	Fruity, green, apple, anise	1.38^{h} ± 0.01	1.45^{i} ± 0.03	4.12^{a} ± 0.01	2.54^{f} ± 0.01	3.77^{b} ± 0.01	2.74^{e} ± 0.01	1.96^{g} ± 0.01
Butanoic acid, hexyl ester		n.d.^{f}	2.67^{a} ± 0.00	2.06^{b} ± 0.01	1.38^{f} ± 0.00	1.39^{f} ± 0.01	1.42^{e} ± 0.01	1.61^{d} ± 0.01
Σ carbonyl compounds		0.72^{i}	0.98^{h}	4.42	2.33	1.84	2.74	3.84
Nonanal	Cut grass	0.10^{f} ± 0.01	0.12^{e,f} ± 0.03	0.70^{a} ± 0.01	0.50^{b} ± 0.01	0.37^{c} ± 0.01	n.d.^{f}	0.17^{e} ± 0.01
Benzaldehyde	Sweet, fruity	n.d.^{e}	n.d.^{e}	n.d.^{e}	n.d.^{e}	0.34^{c} ± 0.01	0.35^{c} ± 0.01	0.55^{a} ± 0.01
2-Pentanone, 5-phenyl-		0.62^{e} ± 0.03	0.34^{f} ± 0.04	2.77^{a} ±0.01	1.21^{b} ± 0.01	0.65^{f} ± 0.01	0.67^{e,f} ± 0.01	0.69^{d,e} ± 0.01
Cyclobutanone, 2-methyl-		n.d.^{f}	0.34^{d} ± 0.01	0.48^{d} ± 0.01	0.45^{e} ± 0.01	0.48^{d} ± 0.01	1.45^{b} ± 0.01	2.04^{a} ± 0.01
3(2H)-Furanone, dihydro-5-isopropyl-	Cotton candy	n.d.^{e}	0.18^{d} ± 0.03	0.47^{a} ± 0.02	0.17^{e} ± 0.01	n.d.^{f}	0.27^{d} ± 0.01	0.39^{c} ± 0.01
Σ fatty acid		n.d.^{i}	20.52^{g}	28.09	13.92	43.95	38.06	57.35
Methyl 4-hydroxybutanoate	Cheese	n.d.^{h}	20.19^{f} ± 0.02	27.64^{e} ± 0.01	13.92^{f} ± 0.01	42.75^{b} ± 0.01	36.71^{d} ± 0.01	42.75^{b} ± 0.01
Butanoic acid, 2-methyl-	Parmesan cheese	n.d.^{c}	n.d.^{c}	n.d.^{c}	n.d.^{c}	0.34^{b} ± 0.01	n.d.^{c}	3.41^{a} ± 0.01
Octanoic acid	Fatty, unpleasant	n.d.^{b}	n.d.^{b}	n.d.^{b}	n.d.^{b}	n.d.^{b}	n.d.^{b}	8.98^{a} ± 0.01
Hexanoic acid	Grass, fruity	n.d.^{g}	0.33^{f} ± 0.01	0.45^{f} ± 0.01	n.d.^{g}	0.87^{d} ± 0.01	1.35^{c} ± 0.01	2.21^{a} ± 0.01
Σ lactones		n.d.^{i}	4.44^{f}	8.28	4.10	7.63	7.22	8.10
γ-Butyrolactone	Sweet, toast, caramel	n.d.^{i}	4.44^{f} ± 0.02	8.28^{a} ± 0.01	4.10^{f} ± 0.01	7.63^{c} ± 0.01	7.22^{d} ± 0.01	8.10^{b} ± 0.01
Σ phenols		n.d.^{i}	5.57^{g}	12.36	10.55	6.52	8.31	12.25
p- Ethylguaiacol	Spicy, coconut	n.d.^{e}	n.d.^{e}	n.d.^{a}	n.d.^{a}	n.d.^{a}	n.d.^{a}	n.d.^{a}
Phenol, 2,6-dimethoxy-	Smoky, leather	n.d.^{b}	n.d.^{b}	1.50^{b} ± 0.01	1.03^{c} ± 0.01	0.00^{e} ± 0.00	n.d.^{e}	1.74^{a} ± 0.01
Benzeneethanol, 4-hydroxy-	Alcohol	n.d.^{i}	5.57^{g} ± 0.03	n.d.^{b}	n.d.^{b}	n.d.^{b}	n.d.^{b}	1.29^{a} ± 0.01
1H-indole-3-ethanol	Alcohol	n.d.^{f}	1.32^{b}	10.86^{a} ± 0.01	9.52^{b} ± 0.02	6.52^{f} ± 0.01	8.31^{d} ± 0.01	9.22^{c} ± 0.01
Σ hydrocarbons		n.d.^{i}	1478.97^{h}	1.37	1.37	1.18	1.05	1.25
1,3-Cyclopentadiene, 5-(1-methylethylidene)-		n.d.^{f}	1.32^{b} ± 0.35	1.37^{b} ± 0.01	1.37^{b} ± 0.01	1.18^{e} ± 0.01	1.05^{f} ± 0.01	1.25^{d} ± 0.0 1
Σ other compounds		n.d.^{e}	n.d.^{e}	n.d.	n.d.	n.d.	0.33	1.72
Diethyl dl-malate	Over ripe, peach, cut grass	n.d.^{e}	n.d.^{e}	n.d.^{e}	n.d.^{e}	n.d.^{e}	0.33^{c} ± 0.02	1.72^{a} ± 0.01
Total		8.06^{i}	1530.00^{h}					

n.d.: Not detected (value under detection limit of 0.5 mg/L); a–i: different letters indicate significant differences between maceration time for the same wine for $P \leq 0.05$.

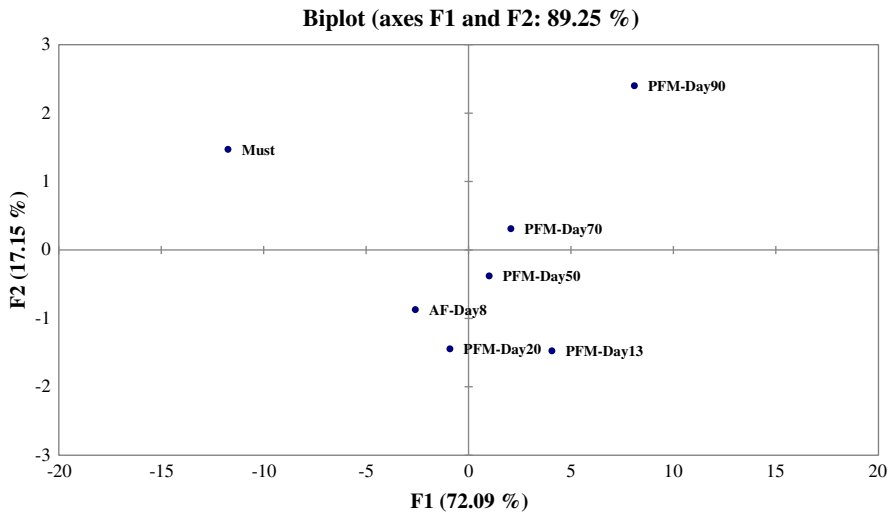

Fig. 3. Score plot for the components F1 and F2 of samples collected during experimental vinification of "Aglianico di Taurasi" wines. Abbreviations: AF, alcoholic fermentation; PFM, post-fermentation maceration. The samples analysed are as follows: AF-Day8, wine on day 8 of alcoholic fermentation; PFM-Day13, wine on day 13 of post-fermentation maceration; PFM-Day20, wine on day 20 of post-fermentation maceration; PFM-Day50, wine on day 50 of post-fermentation maceration; PFM-Day70, wine on day 70 of post-fermentation maceration; PFM-Day90, wine on day 90 of post-fermentation maceration.

positively contribute to fullness, sweetness and roundness sensations (Nurgel and Pickering, 2005). These data support the hypothesis that prolonged maceration could improve the quality of wine due to the activities of yeasts and LAB that are alive and in a metabolically active state during the process.

The results of the phenol analysis confirmed that long maceration improves the quality of the final product. A considerable increase in the phenol compounds was found until the end of the process. In general, maceration favours the extraction of monomeric tannins such as (+)-catechin and (−)-epicatechin that could be responsible for the bitter and acid taste of wine. In fact, maximum concentrations of catechins were found at the end of maceration. This can be explained by the slower extraction kinetics, because they undergo several substitutions and have higher molecular weights (Ribereau-Gayon et al., 2003). On the other hand, it is worth noting that the highest ratio between (+)-catechin and (−)-epicatechin was reached after 40 d. This is an interesting result because epicatechin is more astringent than its chiral isomer (Noble et al., 1999). Thus, extension of the maceration phase up to 40 d may positively contribute to the sensation of roundness of the wine as well as to increased phenol content.

However, with regard to technological, nutritional and sensory properties, catechins represent the most important class of polyphenols and their polymerization with anthocyanins determines the stability of the wine colour (Muñoz et al., 1999).

Our study also showed interesting results in terms of the antioxidant activity of the experimental wines. The highest antioxidant activity was found between days 40 and 50, which corresponded to a high concentration of total polyphenols as well as the highest ratio between (+)-catechin and (−)-epicatechin.

The extended post-fermentation maceration process applied in this work affected the concentration of free radicals. Today, wines with a unique sensory profile and potential health benefits, such as antioxidant activity are particularly requested by the consumers. PCA analysis of VOCs demonstrated that prolonged maceration could greatly influence the characteristics of wine. According to the highest VOC concentrations as well as high sensory scores, 90 day samples appeared clearly separated from those taken at the other collection times and, together with the 70 day samples, they were most distant from the control wine on day 13.

In conclusion, our study provides additional information on the microbial ecology of wine showing that both yeasts and LAB are able to exert metabolic activities even during post-fermentation maceration that is extended to 90 days. Data obtained by chemical and sensory analyses indicated that maceration in the range 40–50 d significantly improves the quality of the final product due to the increase in sensory roundness and complexity as well as the antioxidant activity of wine. In addition, the present study clearly advances our knowledge on the polyphenol content and composition of wines when prolonged maceration is carried out, i.e., longer than the common duration stated by the oenological practice for the production of Aglianico wine. Further investigations on the identification and distribution of LAB species could be useful to allow complete interpretation of the effect of extended maceration on the microbial ecology of wine. Additional experiments carried out with different grape varieties and in different cellars could be useful to advance our knowledge on the wine maceration process.

It is worth noting that, today, maceration processes as the one described here are carried out in large-scale vinifications at private farms and the wines produced according to this winemaking process are commercially distributed and sold in several countries.

Table 5
Sensory scores of "Aglianico di Taurasi" wines carried out by different times of post-fermentation maceration.

Descriptors	Post-fermentation maceration				
	Day-13	Day-20	Day-50	Day-70	Day-90
Colour intensity	5.70^{e}	5.65^{f}	7.89^{a}	7.32^{b}	7.07^{d}
Odour:					
Intensity	6.80^{e}	6.71^{f}	7.31^{b}	6.97^{d}	7.56^{a}
Complexity	5.41^{g}	5.45^{f}	6.86^{b}	6.29^{d}	7.19^{a}
Fresh fruits	5.12^{a}	4.88^{b}	4.03^{d}	3.31^{f}	3.23^{g}
Dried fruits	2.01^{f}	1.84^{g}	2.57^{d}	3.32^{b}	3.82^{a}
Flowers	3.51^{a}	3.16^{b}	2.98^{d}	3.04^{c}	2.77^{f}
Aromatic herbs	1.21^{f}	1.09^{g}	2.15^{d}	2.33^{c}	2.66^{b}
Spices	1.18^{f}	1.11^{g}	2.09^{e}	2.48^{b}	3.15^{a}
Taste:					
Sweet	1.15^{f}	1.09^{g}	1.30^{b}	1.22^{d}	2.12^{a}
Hot	2.21^{f}	1.96^{g}	2.25^{e}	3.28^{b}	3.39^{a}
Acid	4.33^{a}	4.07^{b}	3.51^{d}	3.49^{d}	3.01^{e}
Astringent	7.31^{a}	7.24^{b}	6.97^{c}	6.43^{f}	6.66^{e}
Bitter	5.79^{b}	6.12^{a}	5.35^{d}	5.01^{e}	4.77^{g}
Complexity	4.65^{g}	4.94^{f}	5.81^{c}	5.61^{e}	6.22^{a}

a–g: Different letters indicate significant differences between maceration time for the same sample for $P \leq 0.05$.

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